X-ray Spectromicroscopy of Protein-Polymer Interactions

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INTRODUCTION

Increasingly, medicine uses artificial devices to replace or reinforce diseased body parts or to correct defective body functions. One example is hemodialysis which uses devices made from synthetic polymers. Key problems here and in the many other situations where blood is in contact with artificial surfaces - heart-lung bypass for open heart surgery, artificial heart valves, heart assist devices, arterial grafts, intravascular stents, involving thousands of patients daily worldwide - are activation of blood coagulation, thrombosis, and the immune system. These effects are known to be initiated by interactions of blood proteins with the surface of the material which is often a polymer. The goal of our research is to develop surfaces which prevent or minimize these phenomena [1-3]. Many of the most promising materials have chemically optimized surfaces, others use mechanical structuring, while others use a combination of approaches. Both chemical selectivity and nanostructure are believed to play a role in protein – substrate interactions. Soft X-ray microscopy is a promising tool to apply to biomaterials problems such as this, on account of its high spatial resolution, excellent chemical sensitivity and ability to adapt to a wide range of different situations including vacuum-solid, solid-solid and solid-liquid interfaces. Scanning X-ray transmission microscopy (STXM, BL 7.0.1) and photoelectron emission microscopy (PEEM, BL 7.3.1) are being used to investigate protein interactions with chemically differentiated polymer surfaces. Our experiments in 1999 focussed on determining sensitivity limits and demonstrating correlation of protein location with chemical structure of the polymer substrate.

ALBUMIN ON POLYURETHANE

STXM was first applied to human serum albumin (HSA) *deposited* from an un-buffered aqueous 5 mg/ml solution onto the surface of an ~80 nm polyurethane thin section. C 1s and N 1s STXM images and spectra of the sample were examined before and after exposure. **Figure 1** shows images of the same area of the protein coated polymer at 285.1 ($\pi^*_{C=C}$ of phenyl rings) and 288.2 eV ($\pi^*_{C=O}$ of amide bonds). After subtraction of the spectrum of the underlying polymer, the C 1s spectrum of the albumin coated regions is in good agreement with the C 1s reference spectrum of albumin. Thus NEXAFS readily differentiates the three components – the protein, the PIPA^{TM} (MDI-urea rich) reinforcing particles, and the polyurethane matrix – providing the spectroscopic basis to locate proteins relative to an underlying phase segregated polymer. The critical issue is, can monolayer sensitivity be achieved ?

Encouraged by the clear spectroscopic answer, we next explored detection limits. We found that the C 1s region is more sensitive than the N 1s region since polyurethanes contain nitrogen. N 1s signals have been used successfully for protein imaging in tissue samples with TXM [4], where lower energy resolution precludes use of the strong C 1s $\rightarrow \pi^*_{C=O}$ amide resonance for contrast. It is also useful in PEEM, where the C 1s spectrum is affected by interference from carbonaceous surface contamination. On a Si_3N_4 membrane, where there is no polymer

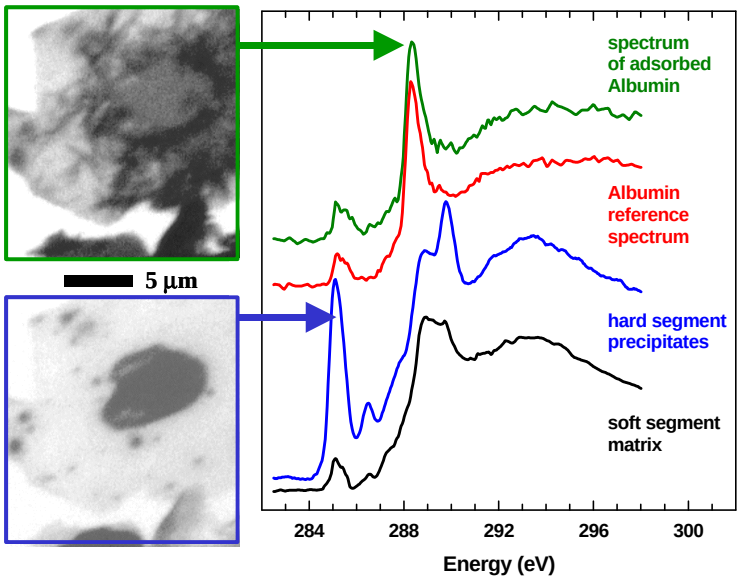

Figure 1 (*upper*) 288.2 eV image of polyurethane thin film exposed to a solution of 5 mg/mL of albumin. (*lower*) 285.1 eV image of the same region. (*right*) Spectra of polymer components (soft and hard regions of the same polymer sample prior to albumin deposition); reference spectrum of an albumin film on a formvar coated TEM grid; and protein spectrum extracted from a ~1 µm^2 region of the protein coated polyurethane by subtraction of appropriately weighted contributions of the polymer component spectra.

background, STXM can easily detect a monolayer of protein [5]. We find that it is possible to detect protein at monolayer levels even against the background C 1s absorption signal of a ~100 nm polymer film. This is demonstrated in **Figure 2**, which presents component maps of matrix, PIPA^{TM}, and albumin from a sample in which albumin was *adsorbed* from dilute solution, with subsequent washing. These maps, derived by pixel-by-pixel curve fits of STXM image sequences [6], report the thickness of each component (in nm, with an error estimated to be ±30%) at each location in the image. In this case, in the regions of lowest coverage, the amount of protein is ~5 nm, about the same as the size of an albumin molecule (~3 x ~8 nm). This rather surprising sensitivity indicates that STXM has the potential to monitor selectivity in adsorption of protein on laterally heterogeneous polymers, and thus has considerable promise for studies of the surfaces of biomaterials.

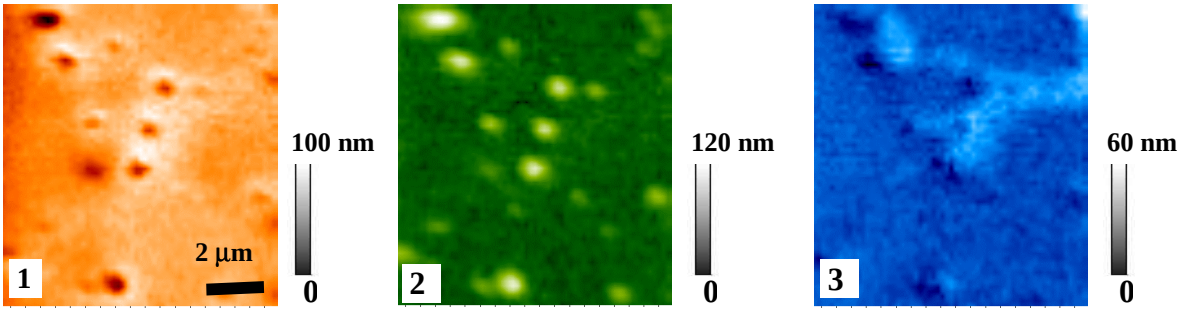

Figure 2 Component maps (1 = matrix, 2 = PIPA^{TM}, 3 = albumin) derived from a regression analysis of a sequence of 86 STXM images (280-315 eV) from a polyurethane with aromatic PIPA^{TM} filler particles which had been exposed to a 1 mg/ml solution of human serum albumin for a few minutes. In each map, the intensity of a given pixel is the mass thickness (ρ.t) derived from the curve fit at that pixel. The spectra for the 3 components are given in Fig. 1. The faintest detectable albumin signal is equivalent to ~5 nm of protein, about a monolayer.

PEEM OF ALBUMIN ON AN AROMATIC-RICH POLYURETHANE

PEEM detects total electron yield and thus is much more surface sensitive than absorption. In addition thin sections are not required. However, PEEM is very sensitive to topography, it frequently has problems with charging for insulating samples like polymers, and it cannot be readily extended to liquid-solid interfaces. We have explored the applicability of PEEM to low

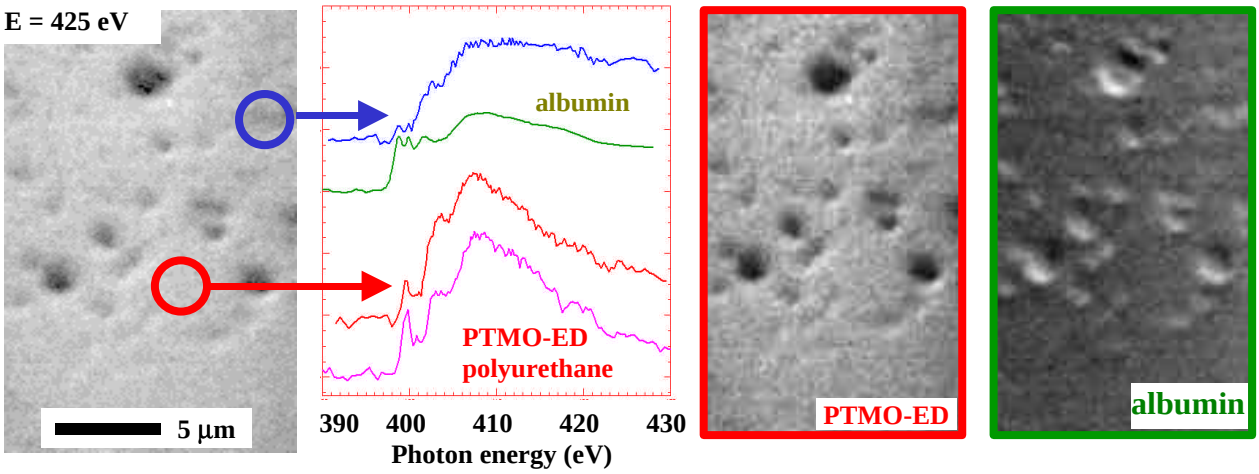

Figure 3. *(left)* PEEM image at 425 eV of a PMTO-ED-MDI polyurethane with monolayer adsorbed human serum albumin protein. *(centre)* Comparison of localized spectra extracted from a N 1s image sequence to those of model spectra recorded with PEEM from pure polymer and pure protein, solution cast onto an unetched Si wafer. *(right* Maps of polyurethane and albumin derived from a PEEM image sequence in the N 1s region

levels of protein adsorbed on polymers to see if its intrinsic surface sensitivity gives significant advantages relative to STXM. **Figure 3** illustrates N 1s regime PEEM detection of human serum albumin adsorbed onto a poly(tetramethyleneoxide)-ethylenediamine-methylenediisocyanate polyurethane (PTMO-ED-MDI) under conditions known to give monolayer adsorption. Although the protein cannot be identified in any single image, the use of regression analyses of image sequences readily maps the protein at very low levels. Further work is needed to quantify the image sequence analysis approach for PEEM, especially since PEEM signals depend on a number of factors including work function [7], which may differ from the pure albumin reference samples to the albumin adsorbed on a polyurethane.

SUMMARY

We have shown that STXM and PEEM spectromicroscopy have analytical sensitivity to protein adsorption on polymers at the monolayer level. Further work is underway to convert the present demonstration experiments into a quantitative tool. This will be applied to systematic studies of protein-polymer selective interaction and interactions of blood proteins such as albumin and fibrinogen with patterned surfaces, steps toward the ultimate goal of helping the development of biomaterials with superior performance in blood contact applications.

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